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Article

Influence of Recombinant Bovine Somatotropin (rbST) on the Metabolic Profile and Milk Composition of Lactating Murrah Buffalo

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Simple Summary: This study was undertaken to evaluate the effects of a single subcutaneous dose of 500 mg of rbST on lipid profile, liver and kidney function and physical, chemical and cellular constitution of buffalo milk. Our data indicate that the application of rbST in buffalo from the 100th day of lactation is metabolically safe, since the treatment neither caused imbalances in fat metabolism nor overloaded the liver or renal function. Changes in milk composition were transient, limited to a decrease in milk protein.

Abstract: The use of recombinant bovine somatotropin (rbST) leads to an increase, in variable amounts, of milk production in buffalo, but there is a lack of information on the influence of rbST on their metabolism. This study looked at the effects of a single 500 mg dose of rbST on lipid profile, liver and kidney function and physical, chemical and cellular constitution of milk in 14 buffalo over 14 days, from the 100th day of lactation, and compared them with 14 animals in a control group. From the 1st day after rbST, there was a rise in beta hydroxybutyrate (β-HBO), possibly due to the higher dry matter intake or by biotransformation of NEFA into β-HBO. Treatment did not influence blood glucose, non-esterified fatty acids (NEFA), triglycerides, cholesterol, total protein, albumin, AST, GGT, bilirubin, urea or creatinine. In 71.3% of the buffalo there was a gradual increase in milk production with maximal response in the first week that was then followed by a gradual decrease, whilst in 21.4%, the increase in production occurred between 7 and 10 days, and only 7.1% of animals did not respond. On the 3rd, 5th, 7th and 10th days after treatment, an increase was found in milk production between the two groups equal to 1.04, 1.52, 1.42 and 1.06 liters, respectively. In relative terms this means an increase in milk production, respectively, of 15.1%; 21.0%; 19.8 % and 15.1 %. The constitution of the milk showed no difference in the amounts of fat, lactose, total solids or somatic cell count; however, on the 3rd day after rbST, there was a decrease in protein; notably, from the 5th day, protein values show no statistical difference. It can be concluded that the application of rbST in buffalo from the 100th day of lactation is metabolically safe, since the treatment neither caused imbalances in fat metabolism nor overloaded the liver or renal function, and the changes in milk composition were transient and limited to a decrease in milk protein.

Keywords: bovine somatotropic hormone; rbST; metabolic parameters; milk yield

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1. Introduction

Buffalo milk has a high nutritional content and great yield for the production of its derivatives, and there has been an increase in the consumption of dairy products from buffaloes in recent decades that has intensified the appreciation of the buffalo species in dairy farming worldwide [1,2]. Given this fact, producers are looking for techniques to increase milk production and the proportion of total solids for industrialization [3]. The administration of Recombinant Bovine Somatotropin (rbST) is a technology that has been important for dairy cattle breeders as it directly impacts the profitability of the system [4].

The use of rbST in female cattle determines gradual increases in milk production a few days after administration, with the maximum response reached within the first week [5], and in addition, there is an increase in lactation persistence, preventing a sharp drop in production after peak [6]. After discontinuation of rbST treatment, there is a gradual decrease in milk production, eventually returning to the levels recorded before the beginning of its use; however, when repeating the treatment every 14 days, the increase in milk production observed in the first week is sustained [5].

Recombinant Bovine Somatotropin is biologically active in sheep [7], goats [8] and buffalo [9]. In buffalo, there was a significant variation in the dosage and interval of repeated application of this hormone, showing an overall increase in milk production between 9.8 and 62.6%, at doses ranging between 320 and 640 mg of rbST repeated every 14, 21 or 28 days [7,9–14].

In studies to assess the influence of rbST in milk composition, alterations in fat and protein were not found when the animals are in a positive energy balance [9–11,15], while somatic cell count showed an enormous variation, which does not allow any statement about the effects of treatment ([10].

The shortage of information about the influence of rbST on the metabolism of buffalo motivated the development of this research, in order to evaluate the effects of a single subcutaneous dose of 500 mg of rbST on lipid profile, liver and kidney function and physical, chemical and cellular constitution of buffalo milk.

2. Materials and Methods

2.1. Animals

A total of 28 healthy female Murrah (Bubalus Bubalis) buffaloes that were in a good body condition, without mammary gland diseases, and with eutocic deliveries and that were between 100 and 200 days of lactation were used in this study. During the experimental period, the animals were kept in a semi-intensive breeding system in Brachiaria ruziziensis pastures; Panicum maximum silage was used as roughage composed of cottonseed (2 Kg/day), brewer residue (10 kg/day) and citrus pulp (2 kg/day). The average composition of the concentrate was 20.8% crude protein and 73% total digestible nutrients (TDN) with an estimated average consumption of 190g of crude protein and 660g of TDN per liter of milk produced. All areas of the study were performed with the approval of Bioethics Committee of the School of Veterinary Medicine and Animal Sciences, University of São Paulo, São Paulo, Brazil (Protocol No. 1405/2008).

In this prospective observational study, two groups of female buffalos were assigned according to the type of treatment submitted. One group consisted of female buffalos (n=14) that received application of recombinant bovine somatotropin (Boostin®, Schering-Plough Animal Health; 500 mg, subcutaneously, in ischiorectal fossa), and a second group (n=14) that consisted of female buffalos that received no of treatment of any kind (Control group).

Females were machine milked twice a day with an average interval of 12 hours between milking. The animals had been averaging a total lactation of 2,000 to 3,000 kg in previous lactations. Milk production was recorded on the day of sampling using the MK-V Waikato Milk Meter.

2.2. Sampling

Blood samples were collected at the following times: immediately before the application of rbST and on 1st, 3rd, 5th, 7th and 14th day after application of rbST.

Blood samples were taken by puncture of the external jugular vein into a tube without anticoagulant (Becton Dickinson Vacutauner® Systems, Frankin Lakes, NJ, USA). The samples were placed in ice and sent to the laboratory within 2 hrs. of collection and were centrifuged at 1,000 × g for 150 min for serum separation. The supernatant serum was stored at −20 ºC until analysis.

The total serum protein was determined by the biuret method; the albumin was tested by the bromocresol green method using Aspartate transaminase (AST), and gamma-glutamyl transpeptidase (GGT), cholesterol and triglycerides were determined using a BioSystems® kit. Bilirubin levels were determined using a Celm® kit. Non-esterified fatty acids (NEFA) and beta hydroxybutyrate (β-HBO) were quantified with a Randox® kit. Blood glucose and urea were determined with a Diasys® kit. Serum creatinine levels were measured using a Labtest® kit. All biochemical determinations were quantified at 25°C in a Biochemical Analyzer Liasys model - AMS - Italy.

For the analysis of physical, chemical and cellular constitution of buffalo milk, 40 ml of milk was collected in plastic vials containing preservative tablets of bronopol (2-bromo-2-nitropropane – 1.3 - diol). Before the start of the somatic cell count and determination of the amounts of lactose, fat, protein and total solids analysis, the milk samples were kept in a water bath at 38°C for 15 minutes, then homogenized manually for the determination of lactose, fat, protein and total solids by infrared radiation using Bentley 2000 equipment (Bentley Instruments Inc). The number of somatic cells in the milk was measured by flow cytometry, using Somacount 500 equipment (Bentley Instruments Inc).

2.3. Statistical methods

In the statistical analysis, the Levene test was used to test the equality hypothesis of the variables. Analysis of variance (ANOVA) was used with repeated measures and multiple comparisons of the least significant difference (equivalent to no adjustment to the significance level). In the ANOVA procedure, the Group, Time and Interaction between Group and Time factors were tested. When the Interaction was significant, the groups were compared separately at each time by the Student T test. For the ANOVA, the significance level was 5%. For multiple comparisons, values of p less than 0.24% were considered (the result of dividing by 5% by 21 comparisons between times 2 to 2).

3. Results

The application of statistical tests could not prove the existence of significant statistical differences between the groups rBST and control ($p = 0.316$); however, when the statistical analysis was performed as a function of the time elapsed after the application of rBST, significant statistical differences were observed ($p<0.001$). It was found in this analysis that only in the group treated with rBST was there an increase in milk production, while in the control group there were no variations in milk production in the studied period (Figure 1). It was observed that milk production in the group treated with rBST was higher in the following moments: day of treatment X 5th day after treatment ($p=0.0019$); day of treatment X 7th day after treatment ($p=0.0020$); 1st day after treatment X 5th day after treatment ($p=0.0009$), and the 1st day after treatment X 10th day after treatment ($p=0.0020$). In 71.3% of the buffalo treated with rBST there was a gradual increase in milk production with maximal response in the first week, followed by a gradual decrease, whilst in 21.4%, the increase in production occurred between 7 and 10 days, and only 7.1% of animals did not respond. On the 3rd, 5th, 7th and 10th days after treatment, an increase was found in milk production between the two groups equal to 1.04, 1.52, 1.42 and 1.06 liters, respectively. In relative terms this means an increase in milk production, respectively, of 15.1%; 21.0%; 19.8 % and 15.1 %.

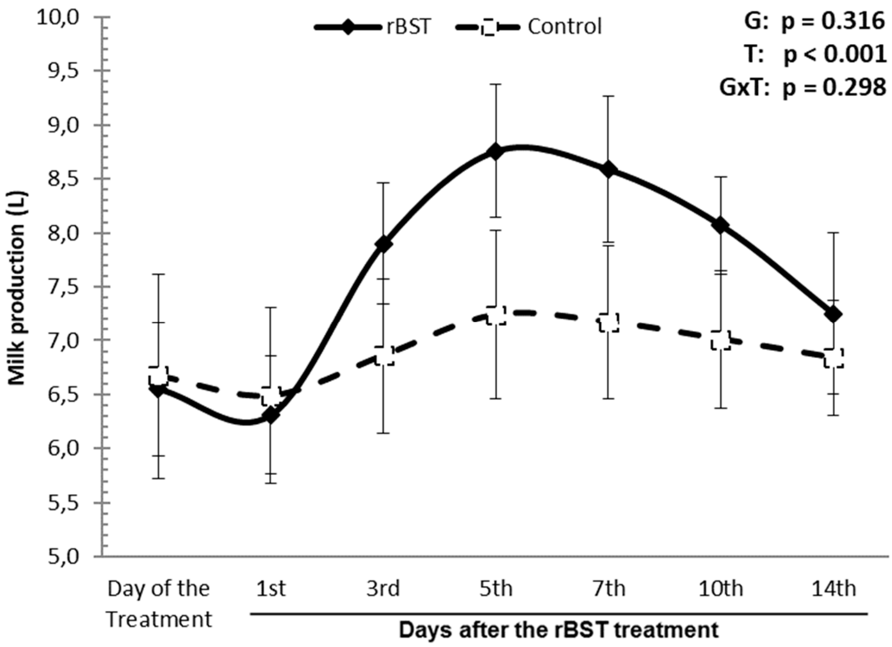


Figure 1. Influence of the application of recombinant bovine somatotropin (rbST) on the amount of milk (L) produced from Murrah buffaloes.

In the early days after the application of rbST, a decrease in milk protein content was observed, and on the 3rd day it was found that milk protein values in the treated group (3.59±0.06 g/dL) were significantly lower than those observed in the control group (3.89±0.06 g/dL). A statistically significant difference was found between the groups on the 3rd day ($p<0.0001$). From the 5th day after the start of hormone treatment, protein values began to oscillate without any statistical difference between the treated and control group. (Figure 2A).

The existence of a relationship between the influence of rbST application on fat, lactose and total solids contents in buffalo milk was not found during the 14 days of study experiment. The fat values, lactose and total solids contents in milk of the group treated with 500 mg of rbST ranged from 4.85±0.30 and 5.53±0.27 g/dL, 4.85±0.13 and 5.02±0.13 g/dL, 14.69±0.29 and 16.02±0.36 g/dL, respectively, while in the control group, the fat, lactose and total solids ranged from between 4.69±0.30 and 5.23±0.34 g/dL, 4.62±0.31 and 4.96±0.07 g/dL, 14.49±0.48 and 15.28±0.33 g/dL, respectively, without any statistical difference being observed during the experimental study (Figure 2B–D).

From the analysis of somatic cell counts in the milk of buffaloes, it was verified the inexistence of the influence of the administration of rbST on the number of somatic cells on the samples collected, whereas there was no statistical difference in the comparison of the experimental groups. During the 14 days of the experiment, the number of somatic cells in milk in the rbST treated group ranged between 253.1±166.9 and 1,113.3±126.6 x 10^3 cells/mL, while in the control group ranged between 171.8±75.1 and 1,457.1±193.7 x 10^3 cells/ mL (Figure 2E).

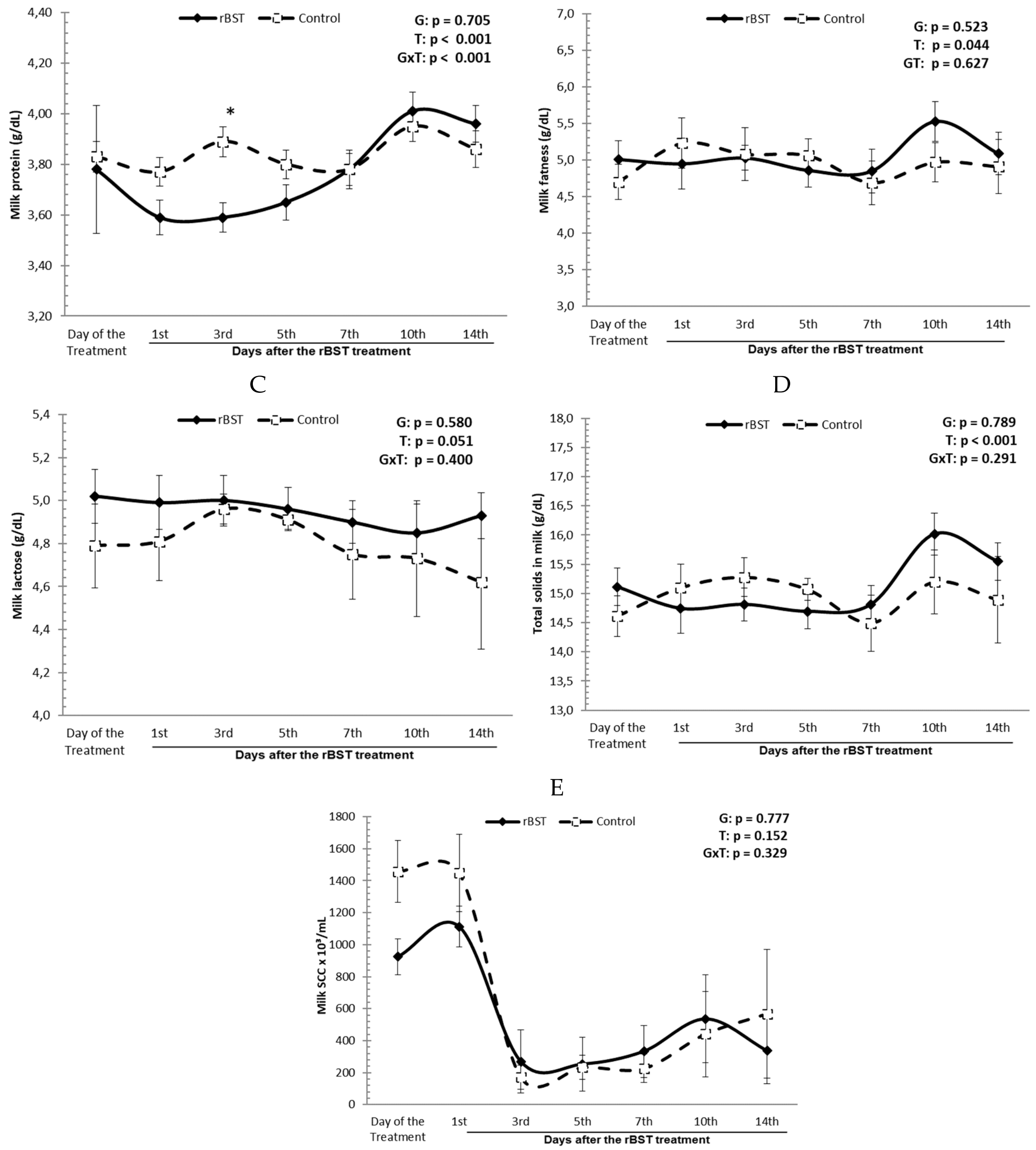


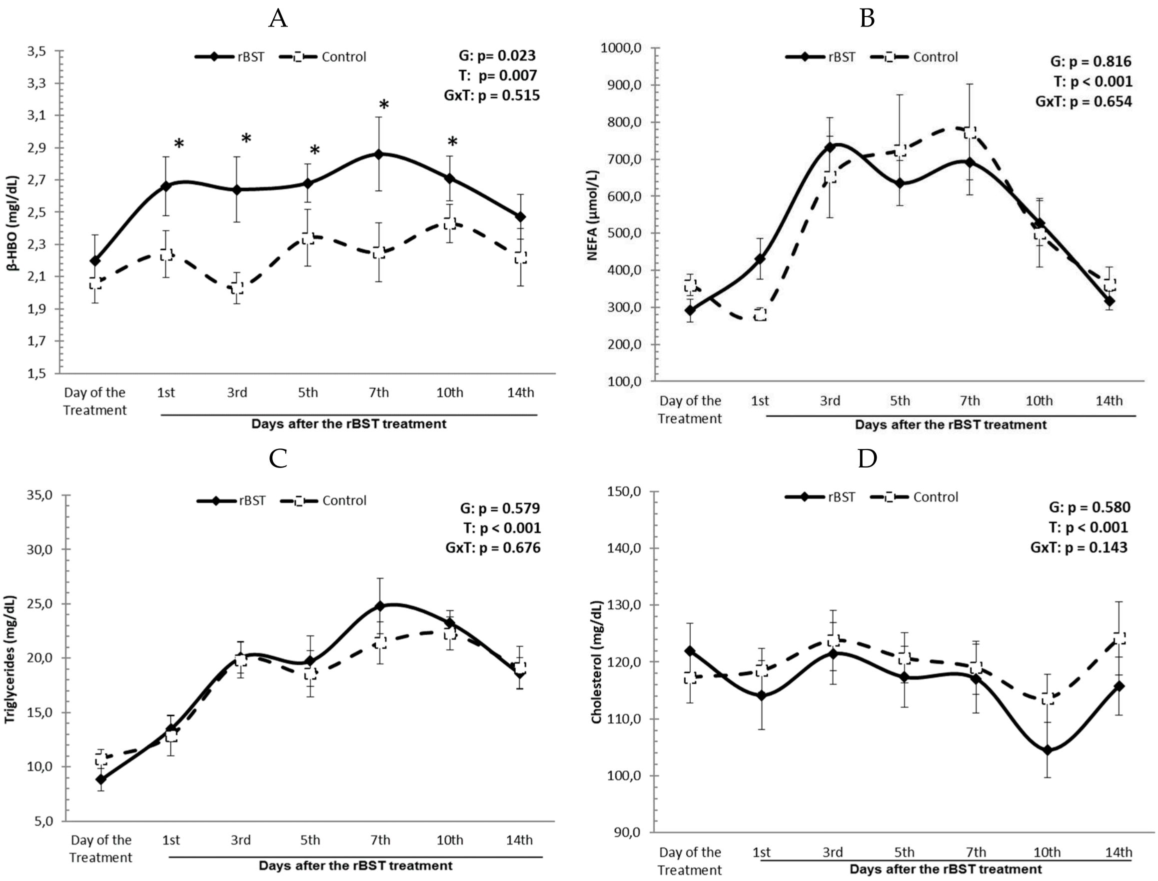
Figure 2. Influence of the application of recombinant bovine somatotropin (rbST) on the physical, chemical and cellular constitution of Murrah buffalo milk: (A) protein milk level; (B) fatness milk level; (C) lactose milk level; (D) total solids milk level and (E) number of somatic cells in the milk – Milk-CCS.

Hormonal treatment with the use of rbST had an effect on the lipidogram of buffaloes in relation to the level of ketone bodies in the blood circulation. Statistically significant difference was observed for β-HBO serum levels between the group treated with 500 mg of rbST and the control group (p=0.0007). From the 1st until 10th days after the hormonal application, the β-HBO serum levels in the treated group were higher (2.64 - 2.86 mmol/L) than those found in the control group (2.03 - 2.43 mmol/L). During this period, the serum levels of β-HBO in the rBST-treated group were between 0.28 and 0.61 mg/dL higher than those found in the control group (Figure 3A).

There was no statistically significant change in NEFA, triglycerides and cholesterol levels due to the hormonal treatment. During the experiment, the serum levels of NEFA in the treated group ranged between 291.6±30.8 and 690.8±87.1 µmol/L, while in the control group they ranged from 281.1±17.7 and 773.7±129.1 µmol/L. The triglycerides serum levels in the group treated with 500 mg of rbST ranged from 8.8±1.0 and 24.8±2.5 mg/dL, while in the control group with no treatment, the values oscillate from 10.7±0.8 and 22.3±1.5 mg/dL. The cholesterol serum levels in the group treated with 500 mg of rbST ranged from 104.5±4.9 and 121.9±4.9 mg/dL, while in the control group the values oscillated from 113.6±4.2 and 124.2±6.4 mg/dL. Also, no influence on plasma blood glucose was observed. The plasmatic glucose concentrations in the group treated with 500 mg of rbST ranged from 64.4±1.6 and 71.6±2.2 mg/dL, while in the control group the values oscillated from 59.4±1.9 and 70.9±1.9 mg/dL

Treatment with the use of rbST had no effect on the liver and renal function of buffaloes (Figure 4). There was no change statistically significant in total protein, albumin, AST, GGT, total bilirubin, urea and creatinine serum levels due to the hormonal treatment. During the experiment, the serum levels of total protein in the treated group ranged between 7.71±0.20 and 8.63±0.14 g/dL, while in the control group they ranged from 7.78±0.16 and 8.65±0.14 g/dL. The albumin serum levels in the group treated with 500 mg of rbST ranged from 3.04±0.06 and 3.61±0.05 g/dL, while in the control group where there was no treatment the values ranged from 3.11±0.10 and 3.66±0.06 g/dL.

The AST serum levels in the group treated with 500 mg of rbST ranged from 114.4±7.3 and 152.0±8.7 U/L, while in the control group the values ranged from 103.8±8.8 and 140.0±5.0 U/L. The GGT serum levels in the group treated with 500 mg of rbST ranged from 25.9±1.6 and 34.8±1.9 U/L, while in the control group the values ranged from 25.9±2.0 and 32.0±2.5 U/L. The serum levels of total bilirubin in the treated group ranged between 0.07±0.01 and 0.32±0.02 mg/dL, while in the control group they ranged from 0.05±0.01 and 0.33±0.02 mg/dL. The serum levels of urea in the treated group ranged between 18.9±1.5 and 40.3±2.4 mg/dL, while in the control group they ranged from 19.6±1.3 and 41.3±1.9 mg/dL. The serum levels of creatinine in the treated group ranged between 1.47±0.03 and 1.70±0.05 mg/dL, while in the control group they ranged from 1.50±0.03 and 1.67±0.04 mg/dL



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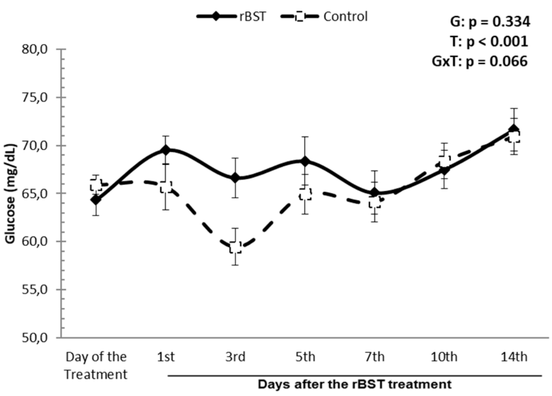
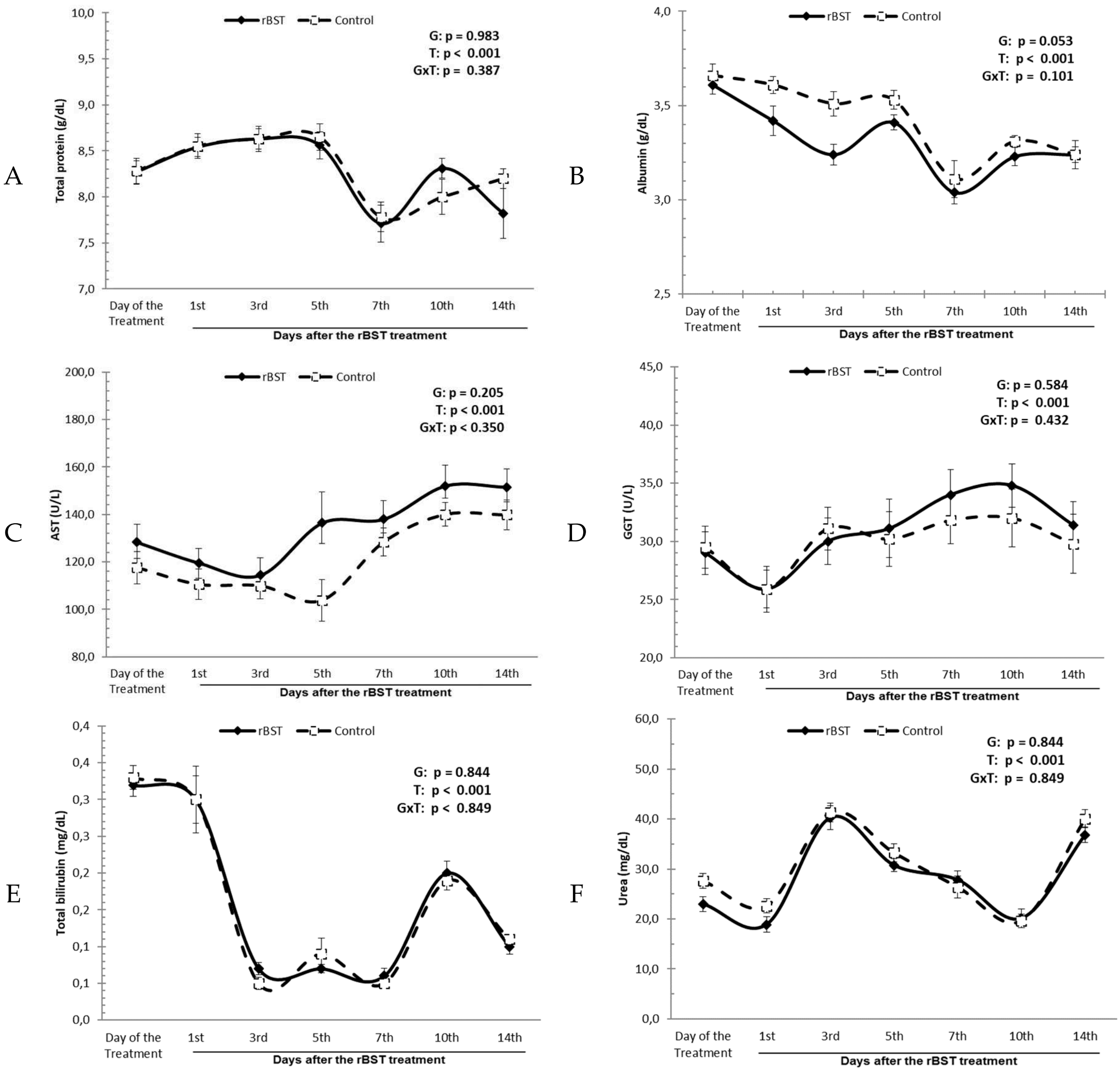


* presence of signal denote the presence of statistical difference between groups.

Figure 3. Influence of the application of recombinant bovine somatotropin (rbST) on the lipidogram and plasmatic glucose concentrations of Murrah buffaloes: (A) β-HBO serum levels, (B) NEFA serum levels, (C) Triglycerides serum levels (D) Cholesterol serum levels (E) Plasmatic glucose concentrations.



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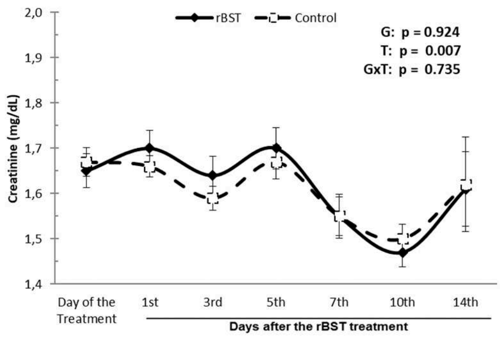

Figure 4. Influence of the application of recombinant bovine somatotropin (rbST) on the liver and renal function of Murrah buffaloes: (A) Total protein serum levels, (B) Albumin serum levels, (C) AST serum levels, (D) GGT serum levels, (E) Total bilirrubin serum levels, (F) Urea serum levels and (G) Creatinine serum levels.

4. Discussion

In this study, it was observed that more than half of the buffaloes had increased milk production in the first week (between 3rd and 5th days) after hormonal application of rbST, while few of animals had increased milk production in the second week (between 7th and 10th days) after hormonal application. The standard response to the use of rbST found in buffalo in this study is similar to that reported for bovine animals: gradual increases in milk production a few days after application, reaching a maximum response during the first week, followed by gradual decrease in milk production [16].

Also in this study the percentual increase in milk production after the hormonal application varied between 15.1% and 21.0%. It was observed that the administration of rbST promotes increases in production that range from 3 to 40% [17]. The impact of the use of rBST in increasing milk production is quite variable as observed in the literature, in which some authors observed significant increases in milk production [11,18,20], while others did not observe an increase in milk production [15,20]. In absolute terms, for the results obtained in our research the increase in milk production varied between 1.0 and 1.5 liters per day. Due the costs of the hormone and the increase in dry matter intake after the application of rBST, it is considered that its use is not economically viable for buffaloes that produce between 6 and 8 liters of milk per day.

The use of rbST make it possible to direct nutrients to the mammary gland through the action of IGF-I, providing an increase in daily milk production and also an increase in total lactation production [21]. It was verified that there was a significant increase in the amount of milk produced in animals that received rbST regardless of the use of this hormone in short or long periods [7,22].

During the evaluation of the lipidogram, it was found that the 1st until 14th days after the application of rbST, the β-HBO serum levels in the treated group were between 0.21 and 0.55 mg/dL higher than those found in the control group. Supplementation with rbST for lactating cows leads to increased demand for nutrients and dry matter intake [23,24]. Thus, the increase in β-HBO levels observed in this study could be a result of increased food ketogenesis due to the increase in dry matter intake and rumen fermentation processes. Another explanation for the increased values of β-HBO is that they could be the result of biotransformation of NEFA into β-HBO by alternative pathway, due to a lack of oxaloacetate.

The effects of rbST on lipid metabolism in buffalo reflect the nutritional and/or physiological conditions of the animals undergoing treatment. In the present study, the results revealed that the lipid metabolism of buffaloes treated with rbST remains unchanged, demonstrating that adaptations of fat metabolism, such as adipose tissue mobilization, oxidation and re-esterification of NEFA in the liver, synthesis of triglycerides and low-density lipoproteins secretion, occurred without overloading lipid metabolism. The lipid metabolism of dairy cows treated with rbST in periods of positive energy balance also did not influence the serum levels of non-esterified fatty acids, triglycerides and cholesterol [25].

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Some authors have reported the occurrence of mild and transient increases in glucose levels in cattle supplemented with rbST, possibly due to increased milk production, with a consequent rise in glucose requirements by the mammary gland for lactose synthesis [21,26–28]. Conversely, other researchers found that the use of rbST does not influence glucose levels in bovine [23,29,30]. In this research, buffalo supplemented with rbST had higher plasmatic glucose concentrations on the 3rd days after treatment; however, these differences were not statistically significant, indicating that metabolic homeostasis is maintained. Thus, it appears that, despite the increase in milk production, with consequent rise in glucose uptake for lactose synthesis in the mammary gland and for the higher metabolism, animals treated with rbST maintained adequate hepatic gluconeogenesis, in order to not interfere with their glycemia.

Adipose tissue also has an important role in glucose concentration, since an increase in the mobilization of adipose tissue leads to an increased availability of glycerol, and consequently, an increased availability of glucogenic precursors [26]. The rbST acts directly on fat metabolism of the adipose tissue by altering rates of lipolysis and lipogenesis [21,31,32]. The use of 500 mg of rbST on the metabolism of pasture-raised Murrah buffaloes, between 63 and 154 days of milking, did not change the levels of other energy metabolites (cholesterol and triglycerides) and prosthetics (total protein, urea albumin and creatinine) in dairy buffaloes [33].

Reports in the literature show the influence of rbST on protein concentration, with the treated animals having an increased total protein [34] and an associated decrease in albumin [27,35] accompanied by increased levels of globulins [35]. There were no indications by the data obtained in this trial that the hepatic function may be harmed by the treatment with rbST. It was observed that total protein, albumin, AST, GGT and bilirubin were not influenced by the rbST treatment.

Urea levels are affected not only by physiological factors, such as diet or state of protein metabolism, but also by changes in renal function [36]. Thus, it is essential to join the values of urea with creatinine, which is an index of glomerular filtration because of their constant excretion and the fact that they are not strongly affected by the nitrogenous compound content in the diet [37]. The occurrence of an energy deficit could have an effect on metabolism, leading to a decrease in the deamination of amino acids and consequently a reduction in serum urea [38,39]. This study did not confirm this possibility, because no changes were observed in serum urea levels.

Regarding the constitution of milk from cows supplemented with rbST, most of the papers did not find changes in their physico-chemical characteristics [21,40,41], and the industrial yield of milk was similar to that obtained from untreated animals [40,42]. In this study, fat values were below that reported in the literature, but they were not affected by rbST treatment, corroborating the papers on buffaloes that did not observe changes in fat content during treatment with rbST [9–11,15]. The explanation for the lack of a direct correction of the administration of rbST on the levels of milk fat is due to the energy status of the animal, since the animals in positive energy balance had no change in the percentage of milk fat different from those of the animals who were in negative energy balance and who had an increase in the concentration of milk fat; the possible reason for the increase is that one of the precursors of this constituent of milk (long chain fatty acids) comes from the lipids circulating in the blood, derived from of diet and adipose tissue mobilized by rbST [43].

In this paper, the influence of rbST on milk protein content was demonstrated. In the early days after the application of rbST, a decrease in milk protein content was observed and on the 3rd day it was found that milk protein values in the treated group were significantly lower than those observed in the control group. From the 5th day after the start of hormone treatment, protein values began to oscillate without any statistical difference between the treated and control group. This result is in line with the results obtained by Bauman [7], who stated that this decline in milk protein rates is due to the time of the greatest gain and response to milk production in cows supplemented with rbST.

Comparing this study with data from the literature, in relation to the concentration of lactose and total solids in milk, the results are in accordance with those reported by several authors [7,15,44], who also did not find any significant difference for these parameters of milk.

The somatic cell count, a final analysis related to the constitution of the milk, is in accordance with that found in cattle [44–46] and in buffaloes [15], since the values for this parameter were not influenced by the rbST treatment.

It can be concluded that rbST application in buffalo from the 100th day of lactation is safe, from a metabolic point of view, since the treatment neither caused imbalances in fat metabolism nor overloaded liver or renal function, and changes in milk composition were transient, limited to a decrease in milk protein. On the other hand, due the costs of the hormone and the increase in dry matter intake after the application of rBST, it is considered that its use is not economically viable for buffaloes that produce between 6 and 8 liters of milk per day

Author Contributions: Marcelo Arne Feckinghaus conceived, designed the studies and performed experiments. Mariana Guimarães de Oliveira Diogo analysed data and wrote the manuscript. Vanessa Martins Storillo analysed data and wrote the manuscript. Fabio Celidonio Pogliani helped in sample collection. Bruno Moura Monteiro helped in drafting the manuscript. Paulo Fantinato Neto helped in analysed data. Melina Marie Yasuoka helped in drafting the manuscript. Daniela Becker Birgel provided the necessary laboratory facilities, and helped in drafting of manuscript. Eduardo Harry Birgel Junior directed and supervised the study, helped in data analysis, and drafting and revising the manuscript.

Data Availability Statement: The corresponding author can provide the data that support the findings of this study upon request.

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Conflicts of Interest: The authors of this study declare that they have no conflicts of interest.

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